

UTILITY PATENT APPLICATION TRANSMITTAL (Large Entity)

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No.
A0000166-66-BD

Total Pages in this Submission
52

TO THE ASSISTANT COMMISSIONER FOR PATENTS

Box Patent Application
Washington, D.C. 20231

Transmitted herewith for filing under 35 U.S.C. 111(a) and 37 C.F.R. 1.53(b) is a new utility patent application for an invention entitled:

**PORCINE ALPHA2DELTA-1 CALCIUM CHANNEL SUBUNIT cDNA AND SOLUBLE SECRETED
ALPHA2DELTA-1 SUBUNIT POLYPEPTIDES**

and invented by:

FRANCOIS BERTELLI, JASON BROWN, VISAKA DISSANAYAKE

If a CONTINUATION APPLICATION, check appropriate box and supply the requisite information:

☐ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior application No.: _____

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Enclosed are:

Application Elements

1. ☒ Filing fee as calculated and transmitted as described below
2. ☒ Specification having 48 pages and including the following:
 - a. ☒ Descriptive Title of the Invention
 - b. ☐ Cross References to Related Applications (if applicable)
 - c. ☐ Statement Regarding Federally-sponsored Research/Development (if applicable)
 - d. ☐ Reference to Microfiche Appendix (if applicable)
 - e. ☒ Background of the Invention
 - f. ☒ Brief Summary of the Invention
 - g. ☐ Brief Description of the Drawings (if drawings filed)
 - h. ☒ Detailed Description
 - i. ☒ Claim(s) as Classified Below
 - j. ☒ Abstract of the Disclosure

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Application Elements (Continued)

3. ☐ Drawing(s) *(when necessary as prescribed by 35 USC 113)*
- a. ☐ Formal Number of Sheets _____
- b. ☐ Informal Number of Sheets _____
4. ☐ Oath or Declaration
- a. ☐ Newly executed *(original or copy)* ☐ Unexecuted
- b. ☐ Copy from a prior application (37 CFR 1.63(d)) *(for continuation/divisional application only)*
- c. ☐ With Power of Attorney ☐ Without Power of Attorney
- d. ☐ DELETION OF INVENTOR(S)
Signed statement attached deleting inventor(s) named in the prior application,
see 37 C.F.R. 1.63(d)(2) and 1.33(b).
5. ☐ Incorporation By Reference *(usable if Box 4b is checked)*
The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied
under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby
incorporated by reference therein.
6. ☐ Computer Program in Microfiche *(Appendix)*
7. ☐ Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all must be included)*
- a. ☐ Paper Copy
- b. ☐ Computer Readable Copy *(identical to computer copy)*
- c. ☐ Statement Verifying Identical Paper and Computer Readable Copy

Accompanying Application Parts

8. ☐ Assignment Papers *(cover sheet & document(s))*
9. ☐ 37 CFR 3.73(B) Statement *(when there is an assignee)*
10. ☐ English Translation Document *(if applicable)*
11. ☐ Information Disclosure Statement/PTO-1449 ☐ Copies of IDS Citations
12. ☐ Preliminary Amendment
13. ☒ Acknowledgment postcard
14. ☒ Certificate of Mailing

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Accompanying Application Parts (Continued)

15. ☐ Certified Copy of Priority Document(s) (if foreign priority is claimed)

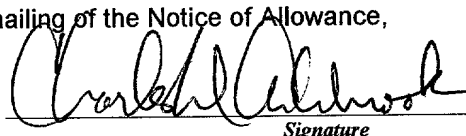
16. ☐ Additional Enclosures (please identify below):

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For	#Filed	#Allowed	#Extra	Rate	Fee
Total Claims	15	- 20 =	0	x \$18.00	\$0.00
Indep. Claims	7	- 3 =	4	x \$78.00	\$312.00
Multiple Dependent Claims (check if applicable) <input type="checkbox"/>					\$0.00
BASIC FEE					\$760.00
OTHER FEE (specify purpose)					
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Signature

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Group Art Unit

Invention: **PORCINE ALPH2DELTA-1 CALCIUM CHANNEL SUBUNIT cDNA AND SOLUBLE SECRETED
ALPHA2DELTA-1 SUBUNIT POLYPEPTIDES**

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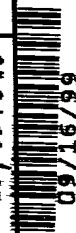
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TITLE OF THE INVENTION: Porcine $\alpha_2\delta$ -1 calcium channel subunit cDNA and soluble secreted $\alpha_2\delta$ -1 subunit polypeptides

5 **BACKGROUND OF THE INVENTION**

Voltage-dependent Ca^{2+} channels (VDCCs) are heteromultimeric complexes present in both neuronal and non-neuronal tissues, including heart and skeletal muscle. VDCCs are minimally composed of three subunits: a pore-forming transmembrane α_1 subunit, a hydrophilic intracellular β subunit, and a membrane-associated $\alpha_2\delta$ -1 subunit; a transmembrane γ subunit is also found in skeletal muscle tissue. Multiple subtypes and/or splice variants of the α_1 , β , and $\alpha_2\delta$ -1 subunits have been found. In heterologous expression studies, the $\alpha_2\delta$ -1 subunit has been shown to increase α_1 currents both by facilitating the assembly of α_1 subunits at the cell surface and by stimulating the peak α_1 current. The modulatory effects of $\alpha_2\delta$ -1 are more pronounced if the α_1 and $\alpha_2\delta$ -1 subunits are co-expressed with the β subunit. However, the functions of the $\alpha_2\delta$ -1, β , and γ subunits in vivo are not yet completely understood.

Gabapentin ((1-aminomethyl)cyclohexane acetic acid or Neurontin) is a structural analogue of GABA, which is mainly used as an adjunctive therapy for epilepsy. Recent research suggests that gabapentin may also have clinical utility for various indications including anxiety and pain. Although designed as a lipophilic GABA-mimetic, gabapentin does not have a high affinity for either GABA_A or GABA_B receptors, GABA uptake sites, or the GABA-degrading enzyme GABA-transaminase (EC 2.6.1.19).

A novel high affinity binding site for [^3H]gabapentin in rat, mouse, and pig brains has been characterized. Recently, the [^3H]gabapentin-binding protein was isolated from pig brain and identified as the $\alpha_2\delta$ -1 subunit of VDCCs. None of the prototypic anticonvulsant drugs displace [^3H]gabapentin binding from the $\alpha_2\delta$ -1 subunit. [^3H]Gabapentin-binding is stereospecifically inhibited by two enantiomers of 3-isobutyl GABA. The rank order of potency of gabapentin, and S- and R-isobutyl GABA, at the [^3H]gabapentin binding site mirrors their anticonvulsant activity in mice. However, electrophysiological studies have yielded conflicting data on the action of gabapentin at VDCCs.

The $\alpha_2\delta$ -1 subunit is derived from a single gene, the product of which is extensively post-translationally modified particularly through the cleavage of the signal sequence. The polypeptide is cleaved to form disulfide-bridged α_2 and δ peptides, both of which are heavily glycosylated. Although the α_2 and δ peptides are membrane-associated peptides, it is unclear whether these peptides comprise one or several transmembrane domains. Furthermore, the location, size and structural configuration of these eventual transmembrane domains remains to be determined.

In any event, the fact that $\alpha_2\delta$ -1 is a membrane-associated protein, regardless of its precise structural configuration, renders its large scale expression in recombinant systems difficult. Indeed, since the $\alpha_2\delta$ -1 protein is targeted to the membrane, it requires detergent solubilisation to purify it. Thus this important drawback imposes considerable restrictions for any potential applications requiring large amounts of recombinant protein.

SUMMARY OF THE INVENTION

In the context of the present invention, the inventors have cloned, isolated and sequenced the porcine cerebral cortical voltage-dependant calcium channel $\alpha_2\delta$ -1 subunit cDNA. (hereinafter the porcine $\alpha_2\delta$ -1 subunit cDNA).

The invention therefore concerns a purified or isolated nucleic acid encoding a porcine $\alpha_2\delta$ -1 subunit cDNA or a sequence complementary thereto.

Oligonucleotide probes or primers specifically hybridizing to a nucleic acid encoding a porcine $\alpha_2\delta$ -1 subunit, to fragments thereof or to a sequence complementary thereto are also part of the invention as well as DNA amplification and detection methods using said primers and probes.

The inventors have also found that it was possible to delete a portion of the nucleotide sequence encoding a eukaryotic, preferably a mammalian $\alpha_2\delta$ -1 subunit to yield a soluble secreted protein which retains its affinity for [3 H]gabapentin and/or other derivatives or compounds such as pregabalin and gabapentoids.

Hence, the invention also concerns nucleotide sequence fragments of an $\alpha_2\delta$ -1 subunit cDNA encoding a soluble secreted $\alpha_2\delta$ -1 subunit polypeptide. Preferably, these nucleotide sequences encode a soluble secreted $\alpha_2\delta$ -1 subunit polypeptide bearing a gabapentin or a [^3H]gabapentin binding site. More preferably, the soluble secreted $\alpha_2\delta$ -1 subunit nucleic acid is derived from the porcine or human $\alpha_2\delta$ -1 subunits.

A further object of the present invention concerns recombinant vectors comprising any of the nucleic acid sequence described herein, and in particular recombinant vectors comprising a nucleic acid sequence encoding a recombinant porcine $\alpha_2\delta$ -1 subunit of the invention.

The invention also includes recombinant vectors comprising a nucleic acid sequence encoding a soluble secreted $\alpha_2\delta$ -1 subunit polypeptide.

The invention also encompasses host cells and transgenic non-human mammals comprising said nucleic acid sequences or recombinant vectors.

The invention concerns an isolated recombinant porcine $\alpha_2\delta$ -1 subunit.

The invention also concerns a porcine $\alpha_2\delta$ -1 subunit polypeptide or a peptide fragment thereof as well as antibodies specifically directed against such porcine $\alpha_2\delta$ -1 subunit polypeptide or peptide fragment.

Furthermore, the invention concerns a secreted soluble $\alpha_2\delta$ -1 subunit polypeptide which is characterized in that it is a soluble secreted polypeptide having affinity for [^3H]gabapentin. Preferably, the soluble secreted polypeptide is derived from the porcine or human $\alpha_2\delta$ -1 subunits.

DETAILED DESCRIPTION OF THE INVENTION

The invention concerns an isolated nucleotide sequence of the porcine $\alpha_2\delta$ -1 subunit cDNA. The invention also concerns truncated $\alpha_2\delta$ -1 subunit cDNA sequences. These truncated sequences encode a soluble secreted polypeptide which retain affinity for

[³H]gabapentin. More details on the various embodiments of the invention are provided below.

A) Porcine $\alpha_2\delta$ -1 subunit cDNA

- 5 A first object of the present invention is of a purified or isolated nucleic acid encoding a porcine $\alpha_2\delta$ -1 subunit, or a sequence complementary thereto.

This cDNA was isolated in several steps. First, a porcine cerebral cortical cDNA library was screened using a fragment of the rabbit skeletal muscle $\alpha_2\delta$ -1 clone as the probe. This allowed the isolation of a $\alpha_2\delta$ -1 coding region which was homologous to the 3' region of
10 the human neuronal $\alpha_2\delta$ -1 sequence but lacked a substantial portion of the 5' coding sequence. The missing sequence was then obtained by 5'-RACE using total RNA prepared from porcine cerebral cortex.

- Another object of the invention is a purified or isolated nucleic acid having at least 90%,
15 preferably 95%, more preferably 98% and most preferably 99% nucleotide identity with the nucleotide sequence of SEQ ID N°1, or a sequence complementary thereto.

- A further object of the present invention is a purified or isolated nucleic acid encoding a polypeptide having at least 80%, preferably 90%, more preferably 95%, and most
20 preferably 98 or 99% amino-acid identity with the porcine polypeptide of the amino-acid sequence of SEQ ID N°5 or with a peptide fragment thereof, or a sequence complementary thereto.

Polypeptides having amino-acid identity with the $\alpha_2\delta$ -1 subunit of the invention encompass polypeptides:

- 25 -that have primary structures which are related to the $\alpha_2\delta$ -1 subunit of any one of the amino-acid sequences of SEQ ID N°5, due to the high sequence identity between the amino-acid sequences; or

- that are biologically related to the polypeptides of any one of the amino-acid sequences of SEQ ID N°5, either because these homologous polypeptides are recognized
30 by antibodies specifically directed against the amino-acid sequence of SEQ ID N°5 and/or because these homologous polypeptides have the same biological activity as the polypeptides of the amino-acid sequence of SEQ ID N°5, such as for example the capacity of binding [³H]gabapentin with suitable affinity.

It is important to note that the first 24 amino acids of the amino acid sequence of SEQ ID N°5 is a signal peptide. This signal peptide can in some embodiments be deleted or replaced by a signal peptide from another species. For example, if one wishes to express this protein in insect cells, the native porcine $\alpha_2\delta$ -1 signal peptide can be replaced by a
5 signal peptide of insect origin.

The term "isolated", when used herein, requires that the material be removed from its original environment (e.g. the natural environment if it is naturally occurring). For example, a naturally occurring polynucleotide or a peptide present in a living animal is not
10 isolated, but the same polynucleotide or peptide, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotide can be part of a vector and/or such polynucleotide or peptide can be part of a composition, and still be isolated. This is so because the vector or composition is not part of the original environment of the nucleotide sequence it contains.

15 The term "purified" does not require absolute purity; rather, it is intended as a relative definition. Purification of starting materials or natural materials to at least one order of magnitude, preferably two or three orders, and more preferably four or five orders of magnitude is expressly contemplated.

Throughout the present specification, the expression "nucleotide sequence" is used to
20 designate indifferently a polynucleotide or a nucleic acid. More precisely, the expression "nucleotide sequence" encompasses the nucleic material and the sequence information and is not restricted to the sequence information (i.e. the succession of letters chosen among the four base letters) that biochemically characterizes a specific DNA or RNA molecule.

As used interchangeably herein, the terms "oligonucleotides", "nucleic acids" and
25 "polynucleotides" include RNA, DNA, or RNA/DNA hybrid sequences of more than one nucleotide in either single chain or duplex form.

Further to its general meaning understood by the one skilled in the art, the term "nucleotide" is also used herein to encompass modified nucleotides which comprise at least one of the following modifications (a) an alternative linking group, (b) an analogous
30 form of purine, (c) an analogous form of pyrimidine, or (d) an analogous sugar. For examples of analogous linking groups, purines, pyrimidines, and sugars, see for example PCT publication N°WO 95/04064.

The polynucleotide sequences of the invention may be prepared by any known method, including synthetic, recombinant, or a combination thereof as well as through any purification methods known in the art.

5 **B) Secreted $\alpha_2\delta$ -1 subunit polypeptides**

The invention also encompasses polynucleotide fragments of a nucleic acid encoding a eukaryotic, preferably a mammal $\alpha_2\delta$ -1 subunit. These fragments particularly include but are not restricted to 1) those fragments encoding a soluble secreted polypeptide of this $\alpha_2\delta$ -1 subunit which preferably retains its binding affinity for [3 H]gabapentin and/or other
10 derivatives or compounds such as pregabalin and gabapentoids and 2) nucleotide fragments useful as nucleic acid primers or probes for amplification or detection purposes. The expression "soluble secreted $\alpha_2\delta$ -1 subunit" is intended to designate polypeptide sequences which, when produced by a recombinant host cell, are secreted at least partially into the culture medium rather than remaining associated with the host cell membrane.

15

1) cDNA fragments encoding soluble secreted $\alpha_2\delta$ -1 subunit polypeptides

One of the important embodiments of the present invention concerns truncated nucleotide sequences of $\alpha_2\delta$ -1 subunit cDNAs which encode soluble secreted $\alpha_2\delta$ -1 subunit polypeptides. The inventors have found that it was possible to generate deletion mutants of
20 $\alpha_2\delta$ -1 subunit cDNAs which, when expressed, produce a significant amount of soluble secreted proteins, preferably soluble secreted proteins, which retain their [3 H]gabapentin binding affinity. These truncated nucleotide sequences of the invention are of significant value to the skilled person as they now allow fast and reliable access to significant concentrations of selected soluble secreted $\alpha_2\delta$ -1 subunit polypeptides. To that end, the
25 inventors have determined the minimal and optimal fragment lengths required to express a polypeptide which: 1) binds [3 H]gabapentin with sufficient affinity and; 2) is obtained in a soluble secreted form.

The discussion provided below provides detailed comments on possible truncations, giving as an example the porcine $\alpha_2\delta$ -1 subunit. However, given the very substantial cross-species
30 homology for $\alpha_2\delta$ -1 subunit sequences, the comments below can also be applied to other eukaryotic species, and more particularly other mammalian species such as rat, mouse, rabbit or human. Their $\alpha_2\delta$ -1 subunit sequences, which are available in public databases, share a very substantial homology with the porcine $\alpha_2\delta$ -1 subunit sequences.

In a first series of experiments, the inventors determined to what extent the coding sequence of the $\alpha_2\delta$ -1 subunit could be truncated and still encode a polypeptide which binds [^3H]gabapentin.

The inventors found that full-length $\alpha_2\delta$ clones expressed in COS cells or in other cells of a similar nature such as HEK cells were partially cleaved by proteolytic enzymes. However, this proteolytic cleavage does not appear to completely separate the α_2 and δ polypeptides encoded by the native gene. In fact, the inventors found that the deletion of the last 7 residues of the δ subunit appears to inhibit proteolytic cleavage of $\alpha_2\delta$ -1. However, mutants on which a portion of the δ subunit coding sequence has been deleted encode proteins which are still binding [^3H]gabapentin even though no proteolytic cleavage seems to occur. Thus, it appears that :

- the $\alpha_2\delta$ -1 polypeptide is not proteolytically cleaved into separate α_2 and δ peptides and;

- at least some of the δ polypeptide must be co-expressed with α_2 to form the [^3H]gabapentin binding pocket.

In order to determine the minimum fragment of the δ subunit required for [^3H]gabapentin binding, the inventors constructed mutants with C-terminal deletions of the δ component. C-terminally truncated mutants extending to residues 966 and 983 of SEQ ID N°5 both do not bind [^3H]gabapentin. However, mutants extending to residues 1018, 1036, 1063 and 1084 of SEQ ID N°5 exhibit gabapentin binding activity. Thus, the inventors have identified a 35-residue stretch between residues 984 to 1018 of SEQ ID N°5 which, when deleted with the C-terminal residues which follow, results in the loss of specific [^3H]gabapentin binding.

Without wishing to be bound by any particular theory, the inventors believe that this region is either directly involved in the formation of the [^3H]gabapentin binding pocket or is required for the structural integrity of the subunit. The two pairs of cysteine residues at positions 984/987 and 1012/1014 may contribute to the tertiary structure of the protein by disulfide bridging. Further deletion experiments on residues 984-1018 of the $\alpha_2\delta$ -1 subunit can be easily carried out by the skilled person to determine which mutants comprising a nucleotide sequence encoding within that region bind [^3H]gabapentin.

In a second series of experiments, the inventors found that nucleotide sequences encoding soluble secreted porcine $\alpha_2\delta$ -1 subunit and which retain their binding affinity for [3 H]gabapentin could be generated by deleting a portion of the $\alpha_2\delta$ -1 subunit cDNA.

In order to determine the optimal deletions on the $\alpha_2\delta$ -1 subunit cDNA that yield a soluble secreted protein devoid of membrane anchorage structures, the inventors tested the expression of several porcine $\alpha_2\delta$ -1 subunit cDNA deletion mutants. The inventors found that by deleting from the porcine $\alpha_2\delta$ -1 subunit cDNA a nucleotide sequence encoding as much as amino-acids 967 to 1091 of the native protein, soluble secreted polypeptides could be obtained. On the other hand, the minimal deletion required to achieve solubility appears to be located around nucleotides encoding amino-acids 1064 to 1091 of the sequence of SEQ ID N°5. In this regard, the mutant polypeptide expressed using a cDNA deletion mutant from which a sequence encoding amino-acids 1064 to 1091 is removed is found in both soluble and membrane-associated forms, with [3 H]gabapentin binding properties similar to that of the wild type protein. Furthermore, a mutant protein expressed using a cDNA deletion mutant from which a nucleotide sequence encoding amino-acids 1085 to 1091 is removed recovers its membrane anchorage properties. Also, mutant proteins expressed using cDNA deletion mutants from which nucleotide sequences encoding either amino-acids 1037 to 1091 or amino-acids 1019 to 1091 of SEQ ID N°5 are removed are found in soluble form.

The inventors believe that the soluble secreted $\alpha_2\delta$ -1 subunit polypeptides which are as close as possible to the native sequence and which are therefore more likely to retain their native folding and hence their [3 H]gabapentin binding properties are those corresponding to the native protein in which amino-acid stretch 985-1091 to 1079-1091 of the amino-acid sequence of SEQ ID N°5 has been deleted. The skilled scientist can quite easily determine within this 90 amino-acid stretch the optimal $\alpha_2\delta$ -1 subunit polypeptides.

The invention therefore particularly concerns a nucleotide sequence encoding a polypeptide having at least 80% identity with the polypeptide comprising from amino-acid 1 to between amino-acids 985 and 1054, preferably between amino-acids 985 and 1059, and most preferably between amino-acids 1019 and 1064 of SEQ ID N°5 or SEQ ID n°14. Preferred nucleotide sequences include those of SEQ ID N°2, SEQ ID N° 3, SEQ ID N°4, SEQ ID n°19, SEQ ID n°20 and SEQ ID n°21.

2) Fragments of the porcine $\alpha_2\delta$ -1 subunit cDNA useful as primers and probes

The present invention also concerns a purified or isolated polynucleotide comprising at least 10 consecutive nucleotides of a nucleic acid encoding the porcine $\alpha_2\delta$ -1 subunit described herein, preferably at least 10 consecutive nucleotides of the nucleotide sequence
5 of SEQ ID N°1, or a sequence complementary thereto.

These nucleic acids consist of a contiguous span which ranges in length from 10, 12, 15, 18 or 20 to 25, 35, 40, 50, 70, 80, 100, 250, 500 or 1000 nucleotides, or be specified as being 10, 12, 15, 18, 20, 25, 35, 40, 50, 100, 200, 250, 500 or 1000 nucleotides in length.

These nucleic acids are useful as probes in order to detect the presence of at least a copy of
10 a nucleotide sequence encoding the porcine $\alpha_2\delta$ -1 subunit, more particularly the presence of at least a copy of a nucleotide sequence of SEQ ID N°1 or a sequence complementary thereto or a fragment or a variant thereof in a sample. The sequence of such nucleic acids could be slightly modified (for example by substituting one nucleotide for another) without substantially affecting the ability of such modified sequence to hybridize with the targeted
15 sequence of interest.

The nucleic acid probes of the invention may also be used for the analysis of the expression levels and patterns of the porcine $\alpha_2\delta$ -1 subunit, such as described in the PCT Application N°WO 97/05 277, the entire contents of which is herein incorporated by
20 reference.

The invention also concerns purified or isolated nucleic acid sequences that hybridize, under stringent hybridization conditions, with a nucleic acid encoding a porcine $\alpha_2\delta$ -1 subunit or a sequence complementary thereto.

As an illustrative embodiment, stringent hybridization conditions can be defined as
25 follows:

The hybridization step is conducted at 65°C in the presence of 6 x SSC buffer, 5 x Denhardt's solution, 0.5 % SDS and 100µg/ml of salmon sperm DNA.

The hybridization step is followed by four washing steps:

- two washings during 5 minutes, preferably at 65°C in a 2 x SSC and 0.1% SDS
30 buffer;
- one washing during 30 minutes, preferably at 65°C in a 2 x SSC and 0.1% SDS buffer;

- one washing during 10 minutes, preferably at 35°C in a 0.1 x SSC and 0.1% SDS buffer,

It being understood that the hybridization conditions defined above are suitable for nucleic acids of approximately twenty nucleotides in length and that these conditions may be also adapted for shorter or longer nucleic acids, according to techniques well known in the art, for example those described by Sambrook et al. (1989).

The appropriate length for probes under a particular set of assay conditions may be empirically determined by the one skilled in the art. The probes can be prepared by any suitable method, including, for example, cloning and restriction of appropriate sequences and direct chemical synthesis by a method such as the phosphodiester method of Narang et al. (1979), the phosphodiester method of Brown et al., (1979), the diethylphosphoramidite method of Beaucage et al. (1981) and the solid support method described in the application N°EP-0 707 792. The disclosures of all these documents are incorporated herein by reference.

Any of the nucleic acids of the present invention can be labelled, if desired, by incorporating a label detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means.

For example, useful labels include radio-active substances (^{32}P , ^{35}S , ^3H , ^{125}I), fluorescent dyes (5-bromodesoxyuridin, fluorecein, acetylaminofluoren, digoxigenin) or biotin. Examples of non-radioactive labelling of nucleic acid fragments are described in French Patent N°FR-78 10975 or by Urdea et al. (1988) or Sanchez-Pescador et al. (1988).

Advantageously, the probes according to the present invention may have structures and characteristics such that they allow signal amplification, such structural characteristics being, for example, those of branched DNA probes as described by Urdea et al. (1991).

Any of the nucleic acid probes of the invention can be conveniently immobilized on a solid support. Solid supports are known those skilled in the art and include the walls of wells of a reaction tray, test tubes, polystyrene beads, magnetic beads, nitro-cellulose strips, membranes, microparticules such as latex particles, sheep red blood cells, duracytes and others.

The nucleic acids of the invention and particularly the nucleotide probes described above can thus be attached to or immobilized on a solid support individually or in groups of at least 2, 5, 8, 10, 12, 15, 20 or 25 distinct nucleic acids of the invention to a single solid support.

- 5 In a specific embodiment of a support on which nucleic acid probes of the invention are immobilized, such a support may also contain other immobilized probes, preferably probes that hybridize specifically with a nucleic acid encoding the porcine $\alpha_2\delta$ -1 subunit, or a variant thereof, or a sequence complementary thereto.

10 **C) Amplification of the porcine $\alpha_2\delta$ -1 subunit cDNA or of soluble secreted $\alpha_2\delta$ -1 subunit nucleotide sequences**

Another object of the invention consists of a method for the amplification of a nucleic acid encoding a porcine $\alpha_2\delta$ -1 subunit or a soluble secreted $\alpha_2\delta$ -1 subunit polypeptide, preferably a polypeptide bearing a [^3H]gabapentin binding site, said method comprising the

15 steps of:

- (a) contacting a test sample suspected of containing the target $\alpha_2\delta$ -1 subunit nucleic acid, a fragment or a variant thereof, or a sequence complementary thereto, with an amplification reaction reagent comprising a pair of amplification primers which can hybridize under stringent conditions, the $\alpha_2\delta$ -1 subunit nucleic acid region to be amplified,
- 20 and
- (b) optionally, detecting the amplification products.

In a first preferred embodiment of the above method, the nucleic acid encodes a porcine $\alpha_2\delta$ -1 subunit of SEQ ID N°5, or a secreted soluble $\alpha_2\delta$ -1 subunit polypeptide of SEQ ID

25 n°6, SEQ ID n°7, SEQ ID n°8, SEQ ID n°15, SEQ ID n°16 and SEQ ID n°17.

In a second preferred embodiment of the above method, a first primer is the nucleotide sequence of SEQ ID N°9 and a second primer is complementary to a portion of the 3' untranslated region of SEQ ID N°5, such as the primer having the sequence of SEQ ID N°22.

- 30 In a third preferred embodiment of the above amplification method, the amplification product is detected by hybridization with a labelled probe having a sequence which is complementary to the amplified region.

The invention also concerns a kit for the amplification of a nucleic acid encoding a porcine $\alpha_2\delta$ -1 subunit, a fragment or a variant thereof, or a complementary sequence thereto in a test sample, wherein said kit comprises:

(a) a pair of oligonucleotide primers which can hybridize, under stringent
5 conditions to $\alpha_2\delta$ -1 subunit nucleic acid to be amplified;

(b) optionally, the reagents necessary for performing the amplification reaction.

In a first preferred embodiment of the kit described above, the nucleic acid encodes the porcine $\alpha_2\delta$ -1 subunit of SEQ ID N°5.

In a second preferred embodiment of the above amplification kit, the amplification product
10 is detected by hybridization with a labelled probe having a sequence which is complementary to the amplified region.

In a third embodiment of the above amplification kit, the amplification primers are respectively the nucleotide sequences of SEQ ID N°9 and SEQ ID N°10.

15 **D) Recombinant vectors and hosts cells for the expression of a porcine $\alpha_2\delta$ -1 subunit or of a secreted soluble $\alpha_2\delta$ -1 subunit polypeptide**

1) Recombinant vectors

The present invention also encompasses a family of recombinant vectors comprising any one of the nucleic acids described herein. Firstly, the invention deals with a recombinant
20 vector comprising a nucleic acid selected from the group consisting of:

(a) a purified or isolated nucleic acid encoding a porcine $\alpha_2\delta$ -1 subunit, and more preferably a polypeptide having at least 80% amino acid identity with the polypeptide of SEQ ID N°5, or a sequence complementary thereto;

(b) a purified or isolated nucleic acid having at least 90% nucleotide identity with a
25 polynucleotide selected from the group consisting of the nucleotide sequences of SEQ ID N°2, SEQ ID N°3, SEQ ID N°4, SEQ ID n°19, SEQ ID n°20 and SEQ ID n°21 or a sequence complementary thereto;

(c) a purified or isolated polynucleotide comprising at least 10 consecutive nucleotides of a nucleic acid described in (a) or a sequence complementary thereto.

30

In a first preferred embodiment a recombinant vector of the invention is used to amplify the inserted polynucleotide derived from the nucleic acid encoding a porcine $\alpha_2\delta$ -1 subunit

of the invention in a suitable host cell, this polynucleotide being amplified every time the recombinant vector replicates.

Recombinant expression vectors comprising a nucleic acid encoding $\alpha_2\delta$ -1 subunit polypeptides that are described in the present specification are also part of the invention.

- 5 These include, but are not restricted to, nucleic acids encoding from amino-acid 1 to between amino-acids 985 to 1054, preferably between amino-acids 984 and 1059, more preferably between amino-acids 1019 to 1064, SEQ ID N°5 and SEQ ID N°14.

- Another preferred embodiment of the recombinant vectors according to the invention consist of expression vectors comprising a nucleic acid encoding an $\alpha_2\delta$ -1 subunit
10 polypeptide of the invention, and more preferably a nucleic acid encoding a polypeptide selected from the group consisting of the amino acid sequences of SEQ ID N°5, SEQ ID N°6, SEQ ID N°7, SEQ ID N°8, SEQ ID n°15, SEQ ID n°16 and SEQ ID n°17.

- Within certain embodiments, expression vectors can be employed to express the porcine
15 $\alpha_2\delta$ -1 subunit of the invention or secreted soluble $\alpha_2\delta$ -1 subunit polypeptides which can then be purified and for example, be used as a immunogen in order to raise specific antibodies directed against said porcine $\alpha_2\delta$ -1 subunit protein or secreted soluble $\alpha_2\delta$ -1 subunit polypeptides.

- Preferred eukaryotic vectors of the invention are listed hereafter as illustrative but not
20 limitative examples: pcDNA3, pFLAG, pCMV-Script, pIND, pMC1NEO, pHIL, pGAPZA, pMT/V5-His-TOPO, pMT/V5-His, pAc5.1/V5-HisA, pDS47/V5-His, pcDNA4, pcDNA6, pEF1, pEF4, pEF6, pUB6, pZeoSV2, pRc/CMv2, pcDM8, pCR3.1, pDisplay, pSecTag2, pVP22, pEMZ, pCMV/Zeo, pSinRep5, pCEP, pREP, pHook-1

- Preferred bacteriophage recombinant vectors of the invention are P1 bacteriophage vectors
25 such as described by Sternberg N.L. (1992;1994).

- A suitable vector for the expression of a porcine $\alpha_2\delta$ -1 subunit polypeptide of the invention or a soluble secreted $\alpha_2\delta$ -1 subunit polypeptide is a baculovirus vector that can be propagated in insect cells and in insect cell-lines. Specific suitable host vectors includes, but are not restricted to pFastBac-1, pIZ/V5-His, pBacMan-1, pBlueBac4.5,
30 pBlueBacHis2, pMelBacA, pVL1392, pVL1393

The recombinant expression vectors from the invention may also be derived from an adenovirus such as those described by Feldman and Steig. (1996) or Ohno et al. (1994).

Another preferred recombinant adenovirus according to this specific embodiment of the present invention is the human adenovirus type two or five (Ad 2 or Ad 5) or an
5 adenovirus of animal origin (French Patent Application n°FR 93 05 954).

a) Regulatory expression sequences

Expression requires that appropriate signals are provided in the vectors, said signals including various regulatory elements such as enhancers/promoters from both viral and
10 mammalian sources that drive expression of the genes of interest in host cells. The regulatory sequences of the expression vectors of the invention are operably linked to the nucleic acid encoding the porcine $\alpha_2\delta$ -1 subunit protein of interest or a soluble secreted $\alpha_2\delta$ -1 subunit polypeptide.

As used herein, the term "operably linked" refers to a linkage of polynucleotide elements
15 in a functional relationship. For instance, a promoter or an enhancer is operably linked to a coding sequence if it affects the transcription of the coding sequence.

More precisely, two DNA molecules (such as a polynucleotide containing a promoter region and a polynucleotide encoding a desired polypeptide or polynucleotide) are said to be "operably linked" if the nature of the linkage between the two polynucleotides does not
20 : (1) result in the introduction of a frame-shift mutation or (2) interfere with the ability of the polynucleotide containing the promoter to direct the transcription of the coding polynucleotide.

Generally, recombinant expression vectors include origins of replication, selectable markers permitting transformation of the host cell, and a promoter derived from a highly
25 expressed gene to direct transcription of a downstream structural sequence. The heterologous structural sequence is assembled in an appropriate frame with the translation, initiation and termination sequences, and preferably a leader sequence capable of directing sequences of the translated protein into the periplasmic space or the extra-cellular medium.

In a specific embodiment wherein the vector is adapted for transfecting and expressing
30 desired sequences in eukaryotic host cells, preferred vectors comprise an origin of replication from the desired host, a suitable promoter and an enhancer, and also any necessary ribosome binding sites, polyadenylation site, transcriptional termination sequences, and optionally 5'-flanking non-transcribed sequences.

DNA sequences derived from the SV 40 viral genome, for example SV 40 origin early promoter, enhancer, and polyadenylation sites may be used to provide the required non-transcribed genetic elements.

5 **b) Promoter sequences**

Suitable promoter regions used in the expression vectors according to the invention are chosen taking into account the host cell in which the heterologous nucleic acids have to be expressed.

A suitable promoter may be heterologous with respect to the nucleic acid for which it controls the expression, or alternatively can be endogenous to the native polynucleotide containing the coding sequence to be expressed.

Additionally, the promoter is generally heterologous with respect to the recombinant vector sequences within which the construct promoter/coding sequence has been inserted.

Preferred eukaryotic promoters are the **(to be completed by inventors)**

15

c) Recombinant host cells

Host cells that have been transformed or transfected with one of the nucleic acids described herein, or with one of the recombinant vector, particularly recombinant expression vector, described herein are also part of the present invention.

20 Are included host cells that are transformed (prokaryotic cells) or are transfected (eukaryotic cells) with a recombinant vector such as one of those described above. Preferred host cells used as recipients for the expression vectors of the invention are the following:

(a) prokaryotic host cells: *Escherichia coli*, strains. (i.e. DH10 Bac strain) *Bacillus subtilis*, *Salmonella typhimurium* and strains from species such as *Pseudomonas*, *Streptomyces* and *Staphylococcus*;

25 (b) eukaryotic host cells: HeLa cells (ATCC N°CCL2; N°CCL2.1; N°CCL2.2), C_v 1 cells (ATCC N°CCL70), COS cells (ATCC N°CRL 1650; N°CRL 1651), Sf-9 cells (ATCC N°CRL 1711), C127 cells (ATCC N°CRL-1804), 3T3 cells (ATCC N°CRL-6361),
30 CHO cells (ATCC N°CCL-61), human kidney 293 cells (ATCC N° 45504; N°CRL-1573), BHK (ECACC N°84100 501; N°84111301), sf 9, sf 21 and hi-5 cells.

E) Production of recombinant $\alpha_2\delta$ -1 subunit polypeptides

The present invention also concerns a method for producing one of the amino acid sequences described herein and especially a polypeptide selected from the group consisting the amino acid sequences of SEQ ID N°5, SEQ ID N°6, SEQ ID N°7, SEQ ID N°8, SEQ ID n°15, SEQ ID n°16 or SEQ ID n°17 wherein said method comprises the steps of:

(a) inserting the nucleic acid encoding the desired amino acid sequence in an appropriate vector;

(b) culturing, in an appropriate culture medium, a host cell previously transformed or transfected with the recombinant vector of step (a);

(c) harvesting the culture medium thus obtained or lyse the host cell, for example by sonication or osmotic shock;

(d) separating or purifying, from said culture medium, or from the pellet of the resultant host cell lysate, the thus produced recombinant polypeptide of interest.

In some instances, it may be required to tag the $\alpha_2\delta$ -1 subunit polypeptide prior to purification. The tag is then in most instances encoded into the nucleotide sequence that is needed to express the polypeptide. Examples of such tags include, but are not limited to sequences encoding C-myc, FLAG, a sequence of histidine residues, heamagglutinin A, V5, Xpress or GST. Most of these tags can be incorporated directly into the sequence, for instance through PCR amplification by incorporating the appropriate coding sequence in one of the PCR amplification primers. However, the tag can also be introduced by other means such as covalent binding of the appropriate nucleic acid sequence encoding the tag moiety with the 3' or 5' end of the nucleic acid sequence encoding the polypeptide sequence. This is the case for GST.

Purification of the recombinant $\alpha_2\delta$ -1 subunit polypeptides according to the present invention is then carried out by passage onto a nickel or copper affinity chromatography column, such as a Ni NTA column.

In another embodiment of the above method, the polypeptide thus produced is further characterized, for example by binding onto an immuno-affinity chromatography column on which polyclonal or monoclonal antibodies directed to the $\alpha_2\delta$ -1 subunit polypeptide, of interest have been previously immobilised.

F) Purified recombinant $\alpha_2\delta$ -1 polypeptides

Another object of the present invention consists of a purified or isolated recombinant polypeptide comprising the amino acid sequence of the porcine $\alpha_2\delta$ -1 subunit or the amino acid sequence of a secreted soluble $\alpha_2\delta$ -1 subunit polypeptide.

5

Preferred isolated recombinant polypeptides of the invention include those having at least 80%, preferably 90%, more preferably 95, and most preferably 98 or 99%, amino-acid identity with polypeptides comprising from amino acid 1 to between amino-acids 985 and 1054, preferably between amino-acids 985 and 1059, and more preferably between amino-

10 acids 1019 and 1064 of SEQ ID N°5 or SEQ ID n°14.

In a further preferred embodiment, the polypeptide comprises an amino acid sequence having at least 80%, preferably 90%, more preferably 95%, and most preferably 98% or 99% amino acid identity with the amino acid sequence of SEQ ID N°5, SEQ ID n°6, SEQ

15 ID N°7, SEQ ID N°8, SEQ ID N°15, SEQ ID N°16 and SEQ ID N°17.

G) Modified $\alpha_2\delta$ -1 subunit polypeptides

The invention also relates to a porcine $\alpha_2\delta$ -1 subunit, or a secreted soluble $\alpha_2\delta$ -1 subunit polypeptide comprising amino acid changes ranging from 1, 2, 3, 4, 5, 10, 20, 25, 30, 35, 40 substitutions, additions or deletions of one amino acid as regards to polypeptides of anyone of the amino acid sequences of the present invention. Preferred sequences are those of SEQ ID N°5, SEQ ID N°6, SEQ ID N°7, SEQ ID N°8, SEQ ID n°15, SEQ ID n°16 and SEQ ID n°17.

25 In the case of an amino acid substitution in the amino acid sequence of a polypeptide according to the invention, one or several consecutive or non-consecutive amino acids are replaced by "equivalent" amino acids. The expression "equivalent" amino acid is used herein to designate any amino acid that may be substituted for one of the amino-acids belonging to the native protein structure without decreasing the binding properties of the

30 corresponding peptides to the antibodies raised against the polypeptides of the invention. In other words, the "equivalent" amino-acids are those which allow the generation or the synthesis of a polypeptide with a modified sequence when compared to the amino acid sequence of the $\alpha_2\delta$ -1 subunit polypeptides of interest, said modified polypeptide being

able to bind to the antibodies raised against the $\alpha_2\delta$ -1 subunit polypeptide of interest and/or to induce antibodies recognizing the parent polypeptide.

Alternatively, amino acid changes encompassed are those which will not abolish the biological activity of the resulting modified polypeptide. These equivalent amino-acids
5 may be determined either by their structural homology with the initial amino-acids to be replaced, by the similarity of their net charge or of their hydrophobicity, and optionally by the results of the cross-immunogenicity between the parent peptides and their modified counterparts.

The peptides containing one or several "equivalent" amino-acids must retain their
10 specificity and affinity properties to the biological targets of the parent protein, as it can be assessed by a ligand binding assay or an ELISA assay.

Examples of amino-acids belonging to specific classes include Acidic (Asp, Glu), Basic (Lys, Arg, His), Non-polar (Ala, Val, Leu, Ile, Pro, Met, Phe, Trp) or uncharged Polar (Gly, Ser, Thr, Lys, Tyr, Asn, Gln) amino-acids.

15 Preferably, a substitution of an amino acid in a porcine $\alpha_2\delta$ -1 subunit polypeptide of the invention, or in a peptide fragment thereof, consists in the replacement of an amino acid of a particular class for another amino acid belonging to the same class.

By an equivalent amino acid according to the present invention is also contemplated the replacement of a residue in the L-form by a residue in the D form or the replacement of a
20 Glutamic acid (E) residue by a Pyro-glutamic acid compound. The synthesis of peptides containing at least one residue in the D-form is, for example, described by Koch (1977).

A specific embodiment of a modified peptide of interest according to the present invention, includes, but is not limited to, a peptide molecule, which is resistant to proteolysis. This is a peptide in which the -CONH- peptide bond is modified and replaced by a (CH₂NH)
25 reduced bond, a (NHCO) retro inverso bond, a (CH₂-O) methylene-oxy bond, a (CH₂S) thiomethylene bond, a (CH₂CH₂) carba bond, a (CO-CH₂) cetomethylene bond, a (CHOH-CH₂) hydroxyethylene bond), a (N-N) bound, a E-alcene bond or also a -CH=CH-bond.

The invention also encompasses a porcine $\alpha_2\delta$ -1 subunit polypeptide or a secreted soluble $\alpha_2\delta$ -1 subunit polypeptide in which at least one peptide bond has been modified as
30 described above.

The polypeptides according to the invention may also be prepared by the conventional methods of chemical synthesis, either in a homogenous solution or in solid phase. As an

illustrative embodiment of such chemical polypeptide synthesis techniques, it may be cited the homogenous solution technique described by Houbenweyl (1974).

The porcine $\alpha_2\delta$ -1 subunit polypeptide of interest, or a fragment thereof may thus be prepared by chemical synthesis in liquid or solid phase by successive couplings of the
5 different amino acid residues to be incorporated (from the N-terminal end to the C-terminal end in liquid phase, or from the C-terminal end to the N-terminal end in solid phase) wherein the N-terminal ends and the reactive side chains are previously blocked by conventional groups.

For solid phase synthesis, the technique described by Merrifield (1965a; 1965b) may be
10 used in particular.

H) Antibody production

The porcine $\alpha_2\delta$ -1 subunit polypeptides of the invention and their peptide fragments of
15 interest can be used for the preparation of antibodies.

Polyclonal antibodies may be prepared by immunization of a mammal, especially a mouse, a rabbit or a sheep, with a polypeptide according to the invention that is combined with an adjuvant of immunity, and then by purifying the specific antibodies contained in the serum of the immunized animal on an affinity chromatography column on which has previously
20 been immobilized the polypeptide that has been used as the antigen.

Monoclonal antibodies may be prepared from hybridomas according to the technique described by Kohler and Milstein (1975).

The present invention also deals with antibodies produced by the trioma technique and by the human B-cell hybridoma technique, such as described by Kozbor et al. (1983).

25 Antibodies of the invention also include chimeric single chain Fv antibody fragments (US Patent N° 4,946,778; Martineau et al., (1998), antibody fragments obtained through phage display libraries Ridder et al. (1995) and humanized antibodies (Leger et al., (1997)).

EXAMPLES

Example 1

Cloning of the Porcine Cerebral Cortical $\alpha_2\delta$ -1 cDNA

An oligo dT-primed λ gt10 porcine cerebral cortical cDNA library was screened by ECL
5 (Amersham) using a 2,381-bp *HindIII* fragment (coding sequence 268-2649) of the rabbit skeletal muscle $\alpha_2\delta$ -1 clone (pcDNA3-Rab- $\alpha_2\delta$ -(+); supplied by Dr. Offord, Parke-Davis Pharmaceutical Research, Ann Arbor, MI) as the probe.

A positive insert was identified and subcloned into pBluescript-SK-(+) to generate pB-PC- $\alpha_2\delta$ -1.1. The clone was sequenced on both strands, except for a 711-bp stretch at one end
10 of the clone, which had a high degree of homology to mitochondrial C oxidase.

The $\alpha_2\delta$ -1 coding region was homologous to the 3' region of the human neuronal $\alpha_2\delta$ sequence but lacked 926 bp of 5' coding sequence. The missing sequence was obtained by 5'-RACE using total RNA prepared from porcine cerebral cortex. RACE was performed
15 across a *BglII* site unique in known $\alpha_2\delta$ -1 sequences (rabbit (accession no. M21948), rat (accession number M86621), human (accession no. M76559)

Primers were designed to amplify the missing 5' portion of the $\alpha_2\delta$ cDNA by 5' Rapid Amplification of cDNA Ends (5' RACE). A series of primers were synthesized based on the $\alpha_2\delta$ cDNA antisense sequence derived from the $\alpha_2\delta$ coding region obtained above, all are downstream (3') of a unique *BglI* restriction site. Total RNA was prepared from porcine
20 cortical membranes and single strand cDNA synthesized using SuperScript II reverse transcriptase and the primer furthest from the *BglI* site (JB039; 5'-TTCTCTAATTCTGCATCAAGG-3', SEQ ID N°24). The cDNA was then purified and tailed with dCTP's using terminal deoxynucleotidyl transferase. Aliquots of this tailing reaction were then PCR amplified through 35 cycles using *Taq* DNA polymerase and the
25 primer pair JB041 (5'-TTTGGATGTAATAAAACATAG-3', SEQ ID N°25) and the universal amplification primer (5'-CUACUACUACUAGGCCACGCGTCGACTAGTAC-3', SEQ ID N°26). Several PCR products were generated and Qiaex gel-purified. All products were positive by Southern blot hybridization using a 1,264bp probe (5' $\alpha_2\delta$ coding sequence) derived from a *HindIII/BglI* restriction digest of pcDNA3-Rab- $\alpha_2\delta$ -(+).
30 Each PCR product was sub-cloned into pBluescript. The 5' and 3' ends of each insert were sequenced confirming that all clones contain $\alpha_2\delta$ sequence as predicted from the Southern blot experiment. The longest of the inserts contained sequence that extended 24bp into the non-coding sequence of the $\alpha_2\delta$ cDNA.

The sequence derived from the 5' RACE product was used to design a primer (JB042, 5'-GGGGATTGATCTTCGATCGCG-3'; SEQ ID N°9) specific for the 5'-untranslated end of the cDNA. PCR was then performed with *Pfu* DNA polymerase using JB042 and a primer downstream of the *Bgl*II site (5'-GCAGATTTGGTTTTAGAAAGGG-3', SEQ ID 22)

- 5 The PCR product was ligated to EcoRI linkers (5'-GGAATTCC-3') and then digested with EcoRI and *Bgl*II. The 1,564-bp fragment (5' portion of the $\alpha_2\delta$ cDNA) was gel-purified.

Similarly, a 2,303-bp fragment (3' portion of the $\alpha_2\delta$ cDNA) was isolated after digestion of pB-PC- $\alpha_2\delta$ -1.1 with *Bgl*II and EcoRI. The two fragments of $\alpha_2\delta$ -1 cDNA were then ligated
10 to EcoRI-digested pcDNA3 in a three-way ligation. A clone was picked with the full-length $\alpha_2\delta$ -1 sequence in the positive orientation with respect to the cytomegalovirus promoter (pcDNA3-PC- $\alpha_2\delta$ (+)). The PCRderived 5' $\alpha_2\delta$ -1 sequence in this plasmid was sequenced on both strands.

15 **Example 2**

Generation and purification of Anti- α_2 and Anti- δ Polyclonal Antibodies

The $\alpha_2\delta$ -1 subunit was purified from porcine brains as described by Gee *et al.* up to, and including, the Sephacryl S400 step. The sample of partially purified $\alpha_2\delta$ -1 subunits was then further purified on a 1-ml CuSO₄ charged iminodiacetic acid-Sepharose column. Prior
20 to each use, the column was recharged with CuSO₄ following a modified version of the protocol described by Brown et al.

Briefly, the column was stripped of metal ions with 3 ml of 0.5 M EDTA/NaOH, pH 8.0 (at 21 °C), washed with 20 ml of H₂O, and then charged with 20 ml of 0.3 M CuSO₄, before a second wash with 20 ml of H₂O and equilibration in buffer A (750 mM NaCl, 0.08%
25 Tween 20, 10 mM HEPES/KOH, pH 7.4 (at 21 °C)).

The partially purified $\alpha_2\delta$ -1 subunits obtained from the S400 chromatography was applied to this column at 0.5 ml/min. Breakthrough material was concentrated to \approx 100 microlitter by ultrafiltration (10,000 M_T cut-off membrane) before separation by SDS-polyacrylamide gel electrophoresis on an 8% preparative gel. The 145-kDa band was excised, and the
30 peptide recovered from the gel by electroelution. Rabbits were immunized by Serotec (Oxford, UK).

Anti- δ antibodies were raised by immunizing rabbits with a keyhole limpet hemocyanin-conjugated peptide, VEMEDDDFTASLSKQSC (SEQ ID N°11), corresponding to the start

sequence of the δ polypeptide (residues 922-938, relative to the first residue of the mature α_2 polypeptide). Peptide synthesis and immunization protocols were performed by Genosys Biotechnologies Inc. (The Woodlands, TX),

- 5 Purified pig brain $\alpha_2\delta$ -1 (125 microgramm) was electrophoresed. under reducing conditions on a single wide track 4-20% gradient SDS-polyacrylamide gel. After transfer onto nitrocellulose membrane, two thin horizontal strips corresponding to the α_2 and δ polypeptides were excised with a razor blade. The strips were incubated with blocking buffer (2% milk powder, 150 mM NaCl, 0.1% Tween 20, 50 mM Tris-Cl, pH 7.5) for 30 min. Immune serum (1 ml) was diluted 5-fold in blocking buffer and incubated with the appropriate strip for 2 h at 4 °C. Strips were then washed three times (15 min each) with blocking buffer and eluted with 2 ml of 50 mM glycine/HCl, pH 2.3. The solution was neutralized with 0.4 ml of 1 M HEPES, pH 8.0. Aliquots of the affinity-purified antibodies were stored frozen at -70°C.

15

Example 3

Construction of C-terminally Deleted Mutant

- For mutants C (Δ 275-1091 (i.e. residues 275 to 1091 deleted)), D (Δ 470-1091), E (Δ 621-1091), F (Δ 804-1091), G (Δ 946-1091), H (Δ 967-1091), I (Δ 984-1091), J (Δ 1019-1091, SEQ ID N°6), K (Δ 1037-1091, SEQ ID N°7), L (Δ 1064-1091, SEQ ID N°8), M (Δ 1085-1091), and PCR-WT (3'-untranslated region deleted) amplifications were performed with an anchored 5' primer (JB055, 5'-TGGCTTATCGAAATTAATACG-3', SEQ ID N°12), which anneals at position 849-869 in pcDNA3-PC- $\alpha_2\delta$ -(+).

- 25 For mutants A (Δ 135-1091) and B (Δ 253-1091), the anchored 5' primer was 5'-AACTCCGGGGATTGATCTTCG-3' (JB115, SEQ ID N°13), which anneals at position 971-991. The 3' primer was designed to anneal internally to the $\alpha_2\delta$ coding sequence to generate the specified C-terminally truncated $\alpha_2\delta$ mutant.

- All 3' primers had the following tail structure: a double stop codon followed by an EcoRI site (5'-CAGAATTCCTCATCA-N₍₁₈₋₂₁₎-3'), where N is the in-frame site-specific sequence complementary to the $\alpha_2\delta$ cDNA. *Pfu* DNA polymerase was used in the PCR reactions a preferred sequence of which is SEQ ID N° 23 (5'-CAGAATTCCTCATCAAGAAACACCAACACAGTCGGT-3') for cloning mutant L,

and the products amplified with JB055 were blunt-end cloned into pBluescript-SK(+). The insert was then subcloned into the EcoRI site of pcDNA3. Products generated with JB115 were cloned directly into the EcoRV site of pcDNA3.

Clones were sequenced to confirm primer regions and a positive orientation with respect to the cytomegalovirus promoter.

Example 4

Construction of a δ -Only Mutant

The α_2 sequence (residues 1-921) was deleted utilizing the two-round PCR method employing *Pfu* DNA polymerase. The product was blunt-end cloned into pBluescriptSK-(+) and then directionally subcloned into pcDNA3 as described above.

Example 5

Transient Expression in COS-7 Cell, extraction of COS-7 membranes and recovery of the soluble fractions

All media contained 50 units/ml penicillin and 50 microgramm per ml streptomycin. COS-7 cells were maintained in Dulbecco's modified Eagle's medium + glutamax, 10% fetal bovine serum (gamma irradiated) in a 37 °C/5% CO₂ incubator and passaged by trypsinization. For transient expression experiments, 150-mm culture dishes were seeded with 3.9×10^6 cells and incubated for 16 h. Cells were then washed twice with 30 ml of optiMEM-1 and transfected (t = 0 h) with 30 microgramm of plasmid DNA by lipofectamine-mediated transfection in 21 ml of optiMEM-1. At t = 6 h, a further 21 ml of optiMEM-1 was added. At t = 24 h, the medium was replaced with 42 ml of optiMEM-1. At t = 48 h, the cells were washed twice with 30 ml of phosphate-buffered saline and then harvested in 3 ml of buffer A (1 nM EDTA, 1mM EGTA, 20% glycerol, 10 mM HEPES, pH 7.4, at 4 °C) plus 0.1 mM phenylmethylsulfonyl fluoride using a cell scraper.

All subsequent operations were performed at 4 °C. The cells were rotated on a Spiramix (Denley Instruments) for 30 min, centrifuged at 20,000 X g for 5 min, resuspended in 1 ml of buffer A, recentrifuged at 20,000 X g for 5 min, and finally resuspended in 400 microliter of buffer A. Membrane preparations were stored at -70 °C until required.

Spent tissue culture medium recovered at t = 24 and 48 h was ultracentrifuged at 100,000 X g for 1 h and then concentrated by ultrafiltration (10,000 M_r cut-off) to approximately 1

ml. The concentrated sample was then extensively dialyzed against buffer A and stored at -70 °C until required.

Samples of membranes (3 microgramms in 48 microliter) were agitated for 2 h on a
5 Spiramix at 4 °C in a total volume of 60 microliters with a final concentration of either 1
M NaCl or 10% ethylene glycol. Samples were ultracentrifuged at 100,000 X g for 2 h, and
20 microliter of supernatant was removed for SDS-polyacrylamide gel electrophoresis.
The pellet was washed again for 10 min at 4 °C in 1 ml of the same buffer before
ultracentrifugation at 100,000 X g for 30 min. The supernatant was discarded, and the
10 pellet was resuspended in 120 microgramms of SDS-polyacrylamide gel electrophoresis
loading buffer and boiled for 20 min; 40 microgramms was loaded onto the gel.

Example 6

Miscellaneous Methods

15 Protein concentrations were determined by the method of Bradford using bovine serum
albumin as a standard. [³H]Gabapentin binding assays were performed as described
previously . For saturation analysis, incubations were performed in duplicate. All other
incubations were performed in triplicate. SDS-polyacrylamide gel electrophoresis and
Western blotting were performed using the Novex gel and buffer system (Novex Europe,
20 Frankfurt, Germany). Molecular weights were determined by reference to Kaleidoscope
markers (Bio-Rad). Detection was performed using the ECL system (Amersham).

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SEQUENCE LISTING

1 - porcine nucleotide sequence alpha2 delta-1

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2 - porcine nucleotide sequence

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5

3 - porcine nucleotide sequence

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20

4 - porcine nucleotide sequence

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35 GTGGTTTGACGCTTAAACTGATCCGAACATCTGTCTCTGAAATGTTGGAAACCCTCTCAGA
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CACCTTGTCCAAGCAAATGTAAGAAATAAGAAAGTGCTGAAAGATGCAGTTAATAATATCA
CAGCAAAAGGAATCACAGATTACAAGAAGGGCTTTAGTTTTGCTTTTGAACAACTGCTTAA

TTATAACGTTTCTAGAGCCAACTGCAATAAGATTATCATGTTGTTACCGATGGAGGAGAA
GAGAGAGCTCAGGAGATATTTGCCAAATACAACAAAGACAAAAAAGTACGTGTATTCACAT
TTTCAGTTGGTCAACATAATTATGACAGAGGACCTATTCAGTGGATGGCCTGTGAAAATAA
AGGTTATTATTATGAAATTCCTTCCATTGGAGCAATCAGAATCAATACTCAGGAATATTTG
5 GATGTTCTGGGAAGACCAATGGTTTTTAGCAGGAGACAAAGCTAAGCAAGTCCAGTGGACAA
ACGTGTACCTGGATGCACTGGAAGTGGGACTTGTCTATTACTGGAAGTCTTCCGGTCTTCAA
CATAACCGGCCAAAATGAAAATAAGACGAACTTAAAGAACCAGCTGATTCTTGGTGTGATG
GGAGTTGATGTATCTTTGGAAGATATTTAAAGACTGACACCACGTTTTTACACTGTGCCCCA
ATGGCTATTACTTTGCAATTGATCCTAATGGCTATGTTTTATTACATCCAAATCTTCAGCC
10 AAAGAACCCCAAATCTCAGGAGCCAGTAACCTTGGATTTCTTGATGCAGAATTAGAGAAT
GATATTAAAGTGGAGATCCGAAATAAAATGATAGATGGAGAAAGTGGAGAAAAACATTCA
GAACTCTGGTTAAATCTCAAGATGAGAGATATATTGACAAAGGAAACAGGACATATACATG
GACTCCTGTCAATGGCACAGATTACAGTTTTGGCCTTGGTATTACCAACCTACAGTTTTTAC
TATATAAAAGCCAAAATAGAAGAGACAATAACTCAGGCCAGATCAAAAAAGGGCAAAATGA
15 AGGATTCAGAAACACTGAAGCCTGATAATTTTGAAGAATCTGGCTATACATTCATAGCACC
AAGAGACTACTGCAATGACCTTAAATATCAGATAATAATACCGAATTTCTTTTAAACTTT
AATGAGTTTATTGATAGAAAACTCCAAACAACCCGTCATGCAACACAGATTTGATTAATA
GAGTCTTGCTGGATGCGGGCTTTACAAATGAACTTGTCCAAAATTACTGGAGTAAGCAGAA
AAACATCAAGGGAGTGAAAGCACGGTTTGTGTAACTGATGGAGGGATTACCAGAGTTTAT
20 CCCAAAGAGGCTGGAGAAAAATTGGCAAGAAAACCCAGAAACATATGAGGACAGCTTCTATA
AAAGAAGTCTAGATAACGATAACTATGTTTTTCACTGCTCCCTACTTTAACAAAAGTGGACC
TGGTGCTTATGAATCAGGCATCATGGTAAGCAAAGCTGTAGAAATATACATCCAAGGAAAA
CTTCTTAAACCTGCAGTTGTTGGAATTTAAATTTGATGTAAATTCCTGGATAGAGAATTTCA
CCAAAACATCAATCAGGGATCCGTGTGCTGGTCCAGTTTGTGATTGTAAAAGAAACAGTGA
25 TGTAATGGATTGTGTGATTCTAGATGATGGTGGGTTTTCTTTTGATGGCAAATCATGATGAT
TATACTAACCAGATTGGAAGGTTTTTTTGGAGAGATTGACCCAAGTTTGATGAGACACCTGG
TTAATATATCAGTTTATGCTTTTAAACAAATCTTACGATTATCAGTCAGTGTGTGAGCCTGG
TGCTGCACCAAAACAAGGAGCAGGACATCGCTCAGCATATGTGCCATCAATAGCAGACATC
TTACACATTGGCTGGTGGGCCACTGCAGCTGCATGGTCTATTCTACAGCAGTTTCTCTTGA
30 GTTTGACCTTTCCACGACTTCTTGAAGCAGTTGAGATGGAAGATGATGACTTTACCGCCTC
TCTGTCAAAGCAGAGTTGCATTACTGAACAAACCCAGTATTTCTTTGATAATGATAGCAAA
TCCTTCAGTGGGGTCTTGGACTGTGGTAACTGTTCCAGAATCTTTCACGTTGAAAAACTTA
TGAACACCAACTTAATATTCATAATGGTTGAGAGCAAAGGGACTTGTCTTGTGACACACG
ATTGCTCATACAAGCTGAGCAGACTTCTGACGGTCCAGATCCTTGTGATATGGTTAAGCAA
35 CCCAGATACCGAAAAGGGCCTGATGTCTGTTTTGATAACAATGCCTTGGAGGATTATACCG
ACTGTGGTGGTGTCTTCTTGA

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5 - porcine amino acid sequence alpha2 delta-1

MAAGCLLALTLTLFQSLIGPSSQEPFPSAVTIKSWVDKMQEDLVTLAKTASGVNQLVDIY
EKYQDLYTVEPNARQLVEIAARDIEKLLSNRSKALVRLALEAEKVQAAHQWREDFASNEV
VYYNAKDDLDPEKNDSEPGSQRIKPVFIDDANFGRQISYQHAAVHIPTDIYEGSTIVLNEL
5 NWTALDEVFKKNREEDPSLLWQVFGSATGLARYYPASPWVDNSRTPNKIDLYDVRRRPWY
IQGAASPKDMLILVDVSGSVSGLTLKLIRTSVSEMLETLSDDDFVNVASFNSNAQDVSCFQ
HLVQANVRNKKVLKDAVNNITAKGITDYKKGFSFAFEQLLNYNVSRANCNKIIMLFTDGGE
ERAQEIFAKYNKDKKVRVFTFSVGQHNYDRGPIQWMACENKGYYYEIPSIGAIRINTQEYL
DVLGRPMVLADGKAKQVQWTNVYLDALGLVITGTLPVFNITGQENKTNLKNQLILGVM
10 GVDVSLEDIKRLTPRFTLCPNGYYFAIDPNGYVLLHPNLQPKNPKSQEPVTLDFLDAELEN
DIKVEIRNKMIDGESGEKTFRTLKVSQDERYIDKGNRTYTWTVPVNGTDYSLALVLPTYSFY
YIKAKIEETITQARSKKGKMKDSETLKPDNFEESGYTFIAPRDYCNLDKISDNNTFLLNF
NEFIDRKTPNNPSCNTDLINRVLLDAGFTNELVQNYWSKQKNIGVKARFVVTDGGITRVY
PKEAGENWQENPETYEDSFYKRLDNDNYVFTAPYFNKSGPGAYESGIMVSKAVEIYIQGK
15 LLKPAVVGIIKIDVNSWIENFTKTSIRDPCAGPVCDCRNSDVMDCVILDDGGFLLMANHDD
YTNQIGRFFGEIDPSLMRHLVNISVYAFNKSVDYQSVCEPGAAPKQGAGHRSAYVPSIADI
LHIGWWATAAAWSILQQFLLSLTFPRLLLEAVEMEDDDFTASLSKQSCITEQTQYFFDNDK
SFSGVLDCGNCSTRIFHVEKLMNTNLIFIMVESKGTCPCDTRLIIQAEQTSDDGPDPCDMVKQ
PRYRKGPDVCFDNNALDYTDGGVSGLNPSLWSIFGIQCVLLWLLSGSRHYQL

20

6- porcine amino acid sequence

MAAGCLLALTLTLFQSLIGPSSQEPFPSAVTIKSWVDKMQEDLVTLAKTASGVNQLVDIY
EKYQDLYTVEPNARQLVEIAARDIEKLLSNRSKALVRLALEAEKVQAAHQWREDFASNEV
VYYNAKDDLDPEKNDSEPGSQRIKPVFIDDANFGRQISYQHAAVHIPTDIYEGSTIVLNEL
25 NWTALDEVFKKNREEDPSLLWQVFGSATGLARYYPASPWVDNSRTPNKIDLYDVRRRPWY
IQGAASPKDMLILVDVSGSVSGLTLKLIRTSVSEMLETLSDDDFVNVASFNSNAQDVSCFQ
HLVQANVRNKKVLKDAVNNITAKGITDYKKGFSFAFEQLLNYNVSRANCNKIIMLFTDGGE
ERAQEIFAKYNKDKKVRVFTFSVGQHNYDRGPIQWMACENKGYYYEIPSIGAIRINTQEYL
DVLGRPMVLADGKAKQVQWTNVYLDALGLVITGTLPVFNITGQENKTNLKNQLILGVM
30 GVDVSLEDIKRLTPRFTLCPNGYYFAIDPNGYVLLHPNLQPKNPKSQEPVTLDFLDAELEN
DIKVEIRNKMIDGESGEKTFRTLKVSQDERYIDKGNRTYTWTVPVNGTDYSLALVLPTYSFY
YIKAKIEETITQARSKKGKMKDSETLKPDNFEESGYTFIAPRDYCNLDKISDNNTFLLNF
NEFIDRKTPNNPSCNTDLINRVLLDAGFTNELVQNYWSKQKNIGVKARFVVTDGGITRVY
PKEAGENWQENPETYEDSFYKRLDNDNYVFTAPYFNKSGPGAYESGIMVSKAVEIYIQGK
35 LLKPAVVGIIKIDVNSWIENFTKTSIRDPCAGPVCDCRNSDVMDCVILDDGGFLLMANHDD
YTNQIGRFFGEIDPSLMRHLVNISVYAFNKSVDYQSVCEPGAAPKQGAGHRSAYVPSIADI
LHIGWWATAAAWSILQQFLLSLTFPRLLLEAVEMEDDDFTASLSKQSCITEQTQYFFDNDK
SFSGVLDCGNCSTRIFHVEKLMNTNLIFIMVESKGTCPCDTRL

7- porcine amino acid sequence

MAAGCLLALTLTLFQSLIGPSSQEPFPSAVTIKSWVDKMQEDLVTLAKTASGVNQLVDIY
EKYQDLYTVEPNARQLVEIAARDIEKLLSNRSKALVRLALEAEKVQAAHQWREDFASNEV
VYYNAKDDLDPEKNDSEPGSQRIKPVFIDANFGRQISYQHAAVHIPTDIYEGSTIVLNEL
5 NWTALDEVFKKNREEDPSLLWQVFGSATGLARYYPASPWVDNSRTPNKIDLYDVRRRPWY
IQGAASPKDMLILVDVSGSVSGLTLKLIRTSVSEMLETLSDDDFVNVASFNSNAQDVSCFQ
HLVQANVRNKKVLKDAVNNITAKGITDYKKGFSFAFEQLLNYNVSRANCNKIIMLFTDGGE
ERAQEIFAKYNKDKKVRVFTFSVGQHNYDRGPIQWMACENKGYYYEIPSIGAIRINTQEYL
DVLGRPMVLAGDKAKQVQWTVNYLDALELGLVITGTLPVFNITGQENKTNLKNQLILGVM
10 GVDVSLEDIKRLTPRFTLCPNGYYFAIDPNGYVLLHPNLQPKNPKSQEPVTLDFLDAELEN
DIKVEIRNKMIDGESGEKTFRTLKVSQDERYIDKGNRTYTWTVPVNGTDYSLALVLPTYSFY
YIKAKIEETITQARSKKGKMKDSETLKPDNFEESGYTFIAPRDYCNDLKISDNNTFLLNF
NEFIDRKTPNNPSCNTDLINRVLLDAGFTNELVQNYWSKQKNIGVKARFVVTDGGITRVY
PKEAGENWQENPETYEDSFYKRSLDNDNYVFTAPYFNKSGPGAYESGIMVSKAVEIYIQGK
15 LLKPAVVGIIKIDVNSWIENFTKTSIRDPCAGPVCDCRNSDVMDCVILDDGGFLLMANHDD
YTNQIGRFFGEIDPSLMRHLVNISVYAFNKSYDYQSVCEPGAAPKQGAGHRSAYVPSIADI
LHIGWWATAAAWSILQQFLLSLTFPRLLLEAVEMEDDDFTASLSKQSCITEQTQYFFDNDK
SFSGVLDCGNCSRIHFHVEKLMNTNLIFIMVESKGTCPCDTRLLIQAEQTS DGPDPDCMVK

20 8 - porcine amino acid sequence

MAAGCLLALTLTLFQSLIGPSSQEPFPSAVTIKSWVDKMQEDLVTLAKTASGVNQLVDIY
EKYQDLYTVEPNARQLVEIAARDIEKLLSNRSKALVRLALEAEKVQAAHQWREDFASNEV
VYYNAKDDLDPEKNDSEPGSQRIKPVFIDANFGRQISYQHAAVHIPTDIYEGSTIVLNEL
NWTALDEVFKKNREEDPSLLWQVFGSATGLARYYPASPWVDNSRTPNKIDLYDVRRRPWY
25 IQGAASPKDMLILVDVSGSVSGLTLKLIRTSVSEMLETLSDDDFVNVASFNSNAQDVSCFQ
HLVQANVRNKKVLKDAVNNITAKGITDYKKGFSFAFEQLLNYNVSRANCNKIIMLFTDGGE
ERAQEIFAKYNKDKKVRVFTFSVGQHNYDRGPIQWMACENKGYYYEIPSIGAIRINTQEYL
DVLGRPMVLAGDKAKQVQWTVNYLDALELGLVITGTLPVFNITGQENKTNLKNQLILGVM
GVDVSLEDIKRLTPRFTLCPNGYYFAIDPNGYVLLHPNLQPKNPKSQEPVTLDFLDAELEN
30 DIKVEIRNKMIDGESGEKTFRTLKVSQDERYIDKGNRTYTWTVPVNGTDYSLALVLPTYSFY
YIKAKIEETITQARSKKGKMKDSETLKPDNFEESGYTFIAPRDYCNDLKISDNNTFLLNF
NEFIDRKTPNNPSCNTDLINRVLLDAGFTNELVQNYWSKQKNIGVKARFVVTDGGITRVY
PKEAGENWQENPETYEDSFYKRSLDNDNYVFTAPYFNKSGPGAYESGIMVSKAVEIYIQGK
LLKPAVVGIIKIDVNSWIENFTKTSIRDPCAGPVCDCRNSDVMDCVILDDGGFLLMANHDD
35 YTNQIGRFFGEIDPSLMRHLVNISVYAFNKSYDYQSVCEPGAAPKQGAGHRSAYVPSIADI
LHIGWWATAAAWSILQQFLLSLTFPRLLLEAVEMEDDDFTASLSKQSCITEQTQYFFDNDK
SFSGVLDCGNCSRIHFHVEKLMNTNLIFIMVESKGTCPCDTRLLIQAEQTS DGPDPDCMVKQ
PRYRKGPDVCFDNNALDYTD CGGVS

9 - nucleic acid sequence

GGGGATTGATCTTCGATCGCG

10 - nucleic acid sequence

5 CTGAGATTTGGGGTTCTTTGG

11- peptide sequence

VEMEDDDFTASLSKQSC

10 12 - nucleic acid sequence

TGGCTTATCGAAATTAATACG

13 - nucleic acid sequence

AACTCCGGGGATTGATCTTCG

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14 - human amino acid sequence alpha2 delta-1

MAAGCLLALTTLTLFQSLIGPSSEEPFPSAVTIKSWVDKMQEDLVTLAKTASGVNQLVDIY
EKYQDLYTVEPNARQLVEIAARDIEKLLSNRSKALVSLALEAEKVQAAHQWREDFASNEV
VYYNAKDDLDPEKNDSEPGSQRIKPVFIEDANFGRQISYQHAAVHIPTDIYEGSTIVLNEL
20 NWTSALEDEVFKKNREEDPSLLWQVFGSATGLARYYPASPWVDNSRTPNKIDLYDVRRRPWY
IQGAASPKDMLILVDVSGSVSGLTLKLIRTSVSEMLETLSDDDFNVNASFNSNAQDVSCFQ
HLVQANVRNKKVLKDAVNNITAKGITDYKKGFSAFAEQLLNYNVSRANCNKIIMLFTDGGE
ERAQEIFNKYNKDKKVRVFRFSVGQHNYERGPIQWMACENKGYYYEIPSIGAIRINTQEYL
DVLGRPMVLAGDKAKQVQWTNVYLDALGLVITGTLPVFNITGQFENKTNLKNQLILGVM
25 GVDVSLEDIKRLTPRFTLCPNGYYFAIDPNGYVLLHPNLQPKNPKSQEPVTLDFLDAELEN
DIKVEIRNKMIDGESGEKTFRTLKVSQDERYIDKGNRTYTWTPVNGTDYSLALVLPITYSYF
YIKAKLEETITQARSKKGKMKDSETLKPDNFEESGYTFIAPRDYCNLDKISDNNTFELLNF
NEFIDRKTPNNPSCNADLINRVLLDAGFTNELVQNYWSKQKNIGVKARFVVTGGITRVY
PKEAGENWQENPETYEDSFYKRSLDNDNYVFTAPYFNKSGPGAYESGIMVSKAVEIYIQGK
30 LLKPAVVGKIDVNSWIENFTKTSIRDPCAGPVCDCKRNSDVMDCVILDDGGFLLMANHDD
YTNQIGRFFGEIDPSLMRHLVNISVYAFNKSXDYQSVCEPGAAPKQGAGHRSAYVPSVADI
LQIGWWATAAAWSILQQFLSLTFPRLLLEAVEMEDDDFTASLSKQSCITEQTQYFFDNDSK
SFSGLVDCGNCSTRIFHGEKLMNTNLIFIMVESKGTCPCDTRLLIQAEQTS DGPNPCDMVKQ
PRYRKGPDPVCFDNNVLEDYTDCCGGVSGLNPSLWYIIGIQFLLLWLVS GSTHRL

35

15 - human amino acid sequence alpha2 delta-1

MAAGCLLALTTLTLFQSLIGPSSEEPFPSAVTIKSWVDKMQEDLVTLAKTASGVNQLVDIY
EKYQDLYTVEPNARQLVEIAARDIEKLLSNRSKALVSLALEAEKVQAAHQWREDFASNEV

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VYYNAKDDLDPEKNDSEPGSQRIKPVFIEDANFGRQISYQHAAVHIPTDIYEGSTIVLNEL
NWTALDEVFKKNREEDPSLLWQVFGSATGLARYYPASPWVDNSRTPNKIDLYDVRRRPWY
IQGAASPKDMLILVDVSGSVSGLTLKLIRTSVSEMLETLSDDDFVNVASFNSNAQDVSCFQ
HLVQANVRNKKVLKDAVNNITAKGITDYKKGFSFAFEQLLNYNVSRANCNKIIMLFTDGGE
5 ERAQEIFNKYNKDKKVRVFRFSVGQHNYERGPIQWMACENKGYYYEIPSIGAIRINTQEYL
DVLGRPMVLAGDKAKQVQWTNVYLDALGLVITGTLPVFNITGQFENKTNLKNQLILGVM
GVDVSLEDIKRLTPRFTLCPNGYYFAIDPNGYVLLHPNLQPKNPKSQEPVTLDFLDAELEN
DIKVEIRNKMIDGESGEKTFRTLKVSQDERYIDKGNRTYTWTPVNGTDYSLALVLPTYSFY
YIKAKLEETITQARSKKGKMKDSETLKPDNFEESGYTFIAPRDYCNDLKISDNNTEFLNLF
10 NEFIDRKTPNNPSCNADLINRVLLDAGFTNELVQNYWSKQKNIGVKARFVVTGIGITRVY
PKEAGENWQENPETYEDSFYKRSLDNDNYVFTAPYFNKSGPGAYESGIMVSKAVEIYIQGK
LLKPAVVGKIDVNSWIENFTKTSIRDPCAGPVCDCRNSDVMDCVILDDGGFLLMANHDD
YTNQIGRFFGEIDPSLMRHLVNISVYAFNKSVDYQSVCEPGAAPKQGAGHRSAYVPSVADI
LQIGWWATAAAWSILQQFLLSLTFPRLLLEAVEMEDDDFTASLSKQSCITEQTQYFFDNDISK
15 SFSGVLDCGNCSRIFHGEKLMNTNLIFIMVESKGTCPCDTRL

16 - human amino acid sequence alpha2 delta-1

MAAGCLLALTTLTLFQSLIGPSSEEPFPSAVTIKSWVDKMQEDLVTAKTASGVNQLVDIY
EKYQDLYTVEPNARQLVEIAARDIEKLLSNRSKALVSLALEAEKVQAAHQWREDFASNEV
20 VYYNAKDDLDPEKNDSEPGSQRIKPVFIEDANFGRQISYQHAAVHIPTDIYEGSTIVLNEL
NWTALDEVFKKNREEDPSLLWQVFGSATGLARYYPASPWVDNSRTPNKIDLYDVRRRPWY
IQGAASPKDMLILVDVSGSVSGLTLKLIRTSVSEMLETLSDDDFVNVASFNSNAQDVSCFQ
HLVQANVRNKKVLKDAVNNITAKGITDYKKGFSFAFEQLLNYNVSRANCNKIIMLFTDGGE
ERAQEIFNKYNKDKKVRVFRFSVGQHNYERGPIQWMACENKGYYYEIPSIGAIRINTQEYL
25 DVLGRPMVLAGDKAKQVQWTNVYLDALGLVITGTLPVFNITGQFENKTNLKNQLILGVM
GVDVSLEDIKRLTPRFTLCPNGYYFAIDPNGYVLLHPNLQPKNPKSQEPVTLDFLDAELEN
DIKVEIRNKMIDGESGEKTFRTLKVSQDERYIDKGNRTYTWTPVNGTDYSLALVLPTYSFY
YIKAKLEETITQARSKKGKMKDSETLKPDNFEESGYTFIAPRDYCNDLKISDNNTEFLNLF
NEFIDRKTPNNPSCNADLINRVLLDAGFTNELVQNYWSKQKNIGVKARFVVTGIGITRVY
30 PKEAGENWQENPETYEDSFYKRSLDNDNYVFTAPYFNKSGPGAYESGIMVSKAVEIYIQGK
LLKPAVVGKIDVNSWIENFTKTSIRDPCAGPVCDCRNSDVMDCVILDDGGFLLMANHDD
YTNQIGRFFGEIDPSLMRHLVNISVYAFNKSVDYQSVCEPGAAPKQGAGHRSAYVPSVADI
LQIGWWATAAAWSILQQFLLSLTFPRLLLEAVEMEDDDFTASLSKQSCITEQTQYFFDNDISK
SFSGVLDCGNCSRIFHGEKLMNTNLIFIMVESKGTCPCDTRLLIQAEQTS DGPNPCDMVK

17 - human amino acid sequence alpha2 delta-1

MAAGCLLALTTLTLFQSLIGPSSEEPFPSAVTIKSWVDKMQEDLVTAKTASGVNQLVDIY
EKYQDLYTVEPNARQLVEIAARDIEKLLSNRSKALVSLALEAEKVQAAHQWREDFASNEV

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VYYNAKDDLDPEKNDSEPGSQRIKPVFIEDANFGRQISYQHAAVHIPTDIYEGSTIVLNEL
NWTSALEDEVFKKNREEDPSLLWQVFGSATGLARYYPASPWVDNSRTPNKIDLYDVRRRPWY
IQGAASPKDMLILVDVSGSVSGLTLKLIRTSVSEMLETLSDDDFVNVASFNSNAQDVSCFQ
HLVQANVRNKKVLKDAVNNITAKGITDYKKGFSFAFEQLLNYNVSRANCNKIIMLFDTGGE
5 ERAQEIFNKYNKDKKVRVFRFSVGQHNYERGPIQWMACENKGYYYEIPSIGAIRINTQEYL
DVLGRPMVLAGDKAKQVQWTNVYLDALGLVITGTLPVFNITGQFENKTNLKNQLILGVM
GVDVSLEDIKRLTPRFTLCPNGYYFAIDPNGYVLLHPNLQPKNPKSQEPVTLDFLDAELEN
DIKVEIRNKMIDGESGEKTFRTLKVSQDERYIDKGNRTYTWTVPVNGTDYSLALVLPTYSFY
YIKAKLEETITQARSKKGKMKDSETLKPDNFEESEGYTFIAPRDYCNLDKISDNNTTEFLNLF
10 NEFIDRKTTPNNPSCNADLINRVLLDAGFTNELVQNYWSKQKNIKGVKARFVVTGCGITRVY
PKEAGENWQENPETYEDSFYKRSLDNDNYVFTAPYFNKSGPGAYESGIMVSKAVEIYIQGK
LLKPAVVGKIDVNSWIENFTKTSIRDPCAGPVCDCKRNSDVMDCVILDDGGFLLMANHDD
YTNQIGRFFGEIDPSLMRHLVNISVYAFNKSVDYQSVCEPGAAPKQGAGHRSAVPSVADI
LQIGWWATAAAWSILQQFLSLTFPRLLLEAVEMEDDDFTASLSKQSCITEQTQYFFDNDISK
15 SFSGLVDCGNCSTRIFHGEKLMNTNLI FIMVESKGTCPCDTRLLIQAEQTS DGPNPCDMVKQ
PRYRKGPDVCFDNNVLEDYTD CGGV S

18 - human nucleic acid sequence alpha2 delta-1

GCGGGGGAGGGGGCATTGATCTTCGATCGCGAAGATGGCTGCTGGCTGCCTGCTGGCCTTG
20 ACTCTGACACTTTTCCAATCTTTGCTCATCGGCCCTCGTCGGAGGAGCCGTTCCCTTCGG
CCGTCACTATCAAATCATGGGTGGATAAGATGCAAGAAGACCTTGTCACACTGGCAAAAAC
AGCAAGTGGAGTCAATCAGCTTGTGTGATATTTATGAGAAATATCAAGATTTGTATACTGTG
GAACCAAATAATGCACGCCAGCTGGTAGAAATTGCAGCCAGGGATATTGAGAAACTTCTGA
GCAACAGATCTAAAGCCCTGGTGAGCCTGGCATTGGAAGCGGAGAAAGTTCAAGCAGCTCA
25 CCAGTGGAGAGAAGATTTTGCAAGCAATGAAGTTGTCTACTACAATGCAAAGGATGATCTC
GATCCTGAGAAAAATGACAGTGAGCCAGGCAGCCAGAGGATAAAACCTGTTTTTCATTGAAG
ATGCTAATTTTGGACGACAAATATCTTATCAGCACGCAGCAGTCCATATTCCTACTGACAT
CTATGAGGGCTCAACAATTGTGTAAATGAACTCAACTGGACAAGTGCCTTAGATGAAGTT
TTCAAAAAGAATCGCGAGGAAGACCCTTCATTATTGTGGCAGGTTTTTGGCAGTGCCACTG
30 GCCTAGCTCGATATTATCCAGCTTCACCATGGGTGATAATAGTAGAACTCCAAATAAGAT
TGACCTTTTATGATGTACGCAGAAGACCATGGTACATCCAAGGAGCTGCATCTCCTAAAGAC
ATGCTTATTCTGGTGGATGTGAGTGGAAAGTGTTAGTGGATTGACACTTAAACTGATCCGAA
CATCTGTCTCCGAAATGTTAGAAACCTCTCAGATGATGATTTTCGTGAATGTAGCTTCATT
TAACAGCAATGCTCAGGATGTAAGCTGTTTTTCAGCACCTTGTCGAAGCAAATGTAAGAAAT
35 AAAAAAGTGTTGAAAGACGCGGTGAATAATATCACAGCCAAAGGAATTACAGATTATAAGA
AGGGCTTTAGTTTTTGCTTTTGAACAGCTGCTTAATTATAATGTTTCCAGAGCAAAGTCAA
TAAGATTATTATGCTATTACGGATGGAGGAGAAGAGAGAGCCAGGAGATATTTAACAAA
TACAATAAAGATAAAAAAGTACGTGTATTACAGTTTTTCAGTTGGTCAACACAATTATGAGA

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GAGGACCTATTTCAGTGGATGGCCTGTGAAAACAAAGGTTATTATTATGAAATTCCTTCCAT
TGGTGCATAAGAATCAATACTCAGGAATATTTGGATGTTTTGGGAAGACCAATGGTTTTTA
GCAGGAGACAAAGCTAAGCAAGTCCAATGGACAAATGTGTACCTGGATGCATTGGAAGTGG
GACTTGTCACTACTGGAAGTCTTCCGGTCTTCAACATAACCGGCCAATTTGAAAATAAGAC
5 AAACCTTAAAGAACCAGCTGATTCTTGGTGTGATGGGAGTAGATGTGTCTTTGGAAGATATT
AAAAGACTGACACCACGTTTTTACACTGTGCCCCAATGGGTATTACTTTGCAATCGATCCTA
ATGGTTATGTTTTATTACATCCAAATCTTCAGCCAAAGAACCCCAAATCTCAGGAGCCAGT
AACATTGGATTTTCCTTGATGCAGAGTTAGAGAATGATATTAAAGTGGAGATTTCGAAATAAG
ATGATTGATGGGGAAAAGTGGAGAAAAAACATTCAGAACTCTGGTTAAATCTCAAGATGAGA
10 GATATATTGACAAAGGAAACAGGACATACACATGGACACCTGTCAATGGCACAGATTACAG
TTTGGCCTTGGTATTACCAACCTACAGTTTTTACTATATAAAAGCCAAACTAGAAGAGACA
ATAACTCAGGCCAGATCAAAAAAGGGCAAAATGAAGGATTCGGAAACCCTGAAGCCAGATA
ATTTTGAAGAATCTGGCTATACATTCATAGCACCAAGAGATTACTGCAATGACCTGAAAAT
ATCGGATAATAACACTGAATTTCTTTTAAATTTCAACGAGTTTTATTGATAGAAAACTCCA
15 AACAACCCATCATGTAACGCGGATTTGATTAATAGAGTCTTGCTTGATGCAGGCTTTACAA
ATGAACTTGTCCAAAATTAAGTGGAGTAAGCAGAAAAATATCAAGGGAGTGAAAGCACGATT
TGTTGTGACTGATGGTGGGATTACCAGAGTTTATCCCAAAGAGGCTGGAGAAAATTGGCAA
GAAAACCCAGAGACATATGAGGACAGCTTCTATAAAAGGAGCCTAGATAATGATAACTATG
TTTTCACTGCTCCCTACTTTAAACAAAAGTGGACCTGGTGCCTATGAATCGGGCATTTATGGT
20 AAGCAAAGCTGTAGAAATATATATTCAAGGGAAACTTCTTAAACCTGCAGTTGTTGGAATT
AAAATTGATGTAAATTCCTGGATAGAGAATTTACCAAAACCTCAATCAGAGATCCGTGTG
CTGGTCCAGTTTGTGACTGCAAAAGAAACAGTGACGTAATGGATTGTGTGATTCTGGATGA
TGGTGGGTTTTCTTCTGATGGCAAATCATGATGATTATACTAATCAGATTGGAAGATTTTTT
GGAGAGATTGATCCCAGCTTGATGAGACACCTGGTTAATATATCAGTTTATGCTTTTAAACA
25 AATCTTATGATTATCAGTCAGTATGTGAGCCCGGTGCTGCACCAAAACAAGGAGCAGGACA
TCGCTCAGCATATGTGCCATCAGTAGCAGACATATTACAAATTGGCTGGTGGGCCACTGCT
GCTGCCTGGTCTATTCTACAGCAGTTTCTCTTGAGTTTGACCTTTCCACGACTCCTTGAGG
CAGTTGAGATGGAGGATGATGACTTCACGGCCTCCCTGTCCAAGCAGAGCTGCATTACTGA
ACAAACCCAGTATTTCTTCGATAACGACAGTAAATCATTCAGTGGTGTATTAGACTGTGGA
30 AACTGTTCCAGAATCTTTCATGGAGAAAAGCTTATGAACACCAACTTAATATTCATAATGG
TTGAGAGCAAAGGGACATGTCCATGTGACACACGACTGCTCATACAAGCGGAGCAGACTTC
TGACGGTCCAAATCCTTGTGACATGGTTAAGCAACCTAGATACCGAAAAGGGCCTGATGTC
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5 ATGGCTGCTGGCTGCCTGCTGGCCTTGACTCTGACACTTTTCCAATCTTTGCTCATCGGCC
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ACTGC

20 - human amino acid sequence alpha2 delta-1

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21- human nucleic acid sequence alpha2 delta-1

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5 GTTTGACCTTTCCACGACTCCTTGAGGCAGTTGAGATGGAGGATGATGACTTCACGGCCTC
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10 CCTAGATACCGAAAAGGGCCTGATGTCTGCTTTGATAACAATGTCTTGAGGATTATACTG
ACTGTGGTGGTGTCTCTG

22 - nucleotide sequence

GCAGATTTGGTTTTAGAAAGG

23 - nucleotide sequence

CAGAATTCCTCATCAAGAAACACCACCACAGTCGGT

24 - nucleotide sequence

TTCTCTAATTCTGCATCAAGG

25 - nucleotide sequence

TTTGGATGTAATAAAACATAG

26 - nucleotide sequence

CUACUACUACUAGGCCACGCGTCGACTAGTAC

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CLAIMS

- 1) A purified or isolated nucleic acid encoding a eukaryotic secreted soluble cerebral cortical voltage-dependent calcium channel $\alpha_2\delta$ -1 subunit polypeptide.
- 5 2) A purified or isolated nucleic acid according to claim 1, comprising a polynucleotide having at least 90% identity with the sequence encoding from amino-acid 1 to between amino-acids 1009 to 1083 of SEQ ID N°5 or SEQ ID n°14.
- 3) A purified or isolated nucleic acid according to claim 1, having at least 90% identity with the sequence encoding from amino-acid 1 to between amino-acids 1043 and 1088
10 of SEQ ID N°5 or SEQ ID n°14.
- 4) A purified or isolated nucleotide sequence according to claim 1 wherein said sequence is the sequence of SEQ ID N°2, SEQ ID n°3, SEQ ID n°4, SEQ ID n°19, SEQ ID n°20 or SEQ ID n°21.
- 5) A purified or isolated nucleic acid, having at least 90% identity with the nucleotide
15 sequence of SEQ ID N°1.
- 6) A purified or isolated polynucleotide comprising at least 10 consecutive nucleotides of the nucleotide sequence of SEQ ID N°1.
- 7) A polynucleotide probe or primer hybridizing, under stringent conditions, with the nucleic acid according to claim 5.
- 20 8) A method for the amplification of a nucleic acid encoding a eukaryotic secreted soluble cerebral cortical voltage-dependent calcium channel $\alpha_2\delta$ -1 subunit polypeptide, said method comprising the steps of:
 - (a) contacting a test sample suspected of containing the target secreted soluble $\alpha_2\delta$ -1 subunit nucleic acid, or a sequence complementary thereto, with an amplification
25 reaction reagent comprising a pair of amplification primers located on either side of the $\alpha_2\delta$ -1 subunit nucleic acid region to be amplified, and
 - (b) optionally, detecting the amplification products.
- 9) A kit for the amplification of a nucleic acid encoding a secreted soluble $\alpha_2\delta$ -1 subunit
30 polypeptide, or a complementary sequence thereto in a test sample, wherein said kit comprises:

(a) a pair of oligonucleotide primers which can hybridize, under stringent conditions, to the secreted soluble $\alpha_2\delta$ -1 subunit nucleic acid region to be amplified;

(b) optionally, the reagents necessary for performing the amplification reaction.

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10) A recombinant vector comprising a nucleic acid according to claim 1.

11) A recombinant host cell comprising a nucleic acid according to claim 1.

12) A method for producing a secreted soluble $\alpha_2\delta$ -1 subunit wherein said method comprises the steps of:

10 (a) inserting the nucleic acid encoding the desired $\alpha_2\delta$ -1 subunit polypeptide in an appropriate vector;

(b) culturing, in an appropriate culture medium, a host cell previously transformed or transfected with the recombinant vector of step (a);

15 (c) harvesting the culture medium thus obtained or lyse the host cell, for example by sonication or osmotic shock;

(d) separating or purifying, from said culture medium, or from the pellet of the resultant host cell lysate, the thus produced $\alpha_2\delta$ -1 subunit polypeptide of interest.

20 13) A purified or isolated recombinant polypeptide comprising the amino acid sequence of a secreted soluble $\alpha_2\delta$ -1 subunit polypeptide.

14) A recombinant polypeptide according to claim 13, having at least 80% amino acid identity with a polypeptide comprising from amino acid 1 to between amino acids 985 and 1054 of the amino acid sequence of SEQ ID N°5 or SEQ ID n°14, or a sequence complementary thereto.

25 15) A recombinant polypeptide according to claim 14, wherein said recombinant polypeptide is selected from the group consisting of the amino acid sequences of SEQ ID n°6, SEQ ID n°7, SEQ ID n°8, SEQ ID n°15, SEQ ID n°16 and SEQ ID n°17.

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ABSTRACT

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Porcine $\alpha_2\delta$ -1 calcium channel subunit cDNA and soluble secreted $\alpha_2\delta$ -1 subunit polypeptides

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Soluble $\alpha_2\delta$ -1 subtype polypeptides.

15 Methods for cloning, expression and purification of freely soluble $\alpha_2\delta$ -1 subtype polypeptides.

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